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Keywords

Bile duct; cholangiocyte; cholangiopathy; drug-induced liver injury; idiosyncrasy; vanishing bile duct syndrome

Abbreviations

DILI, Drug-Induced Liver Injury; DILIN, Drug-Induced Liver Injury Network; HLA, Human Leukocyte Antigen; PBC, Primary Biliary Cholangitis; PSC, Primary Sclerosing Cholangitis; RUCAM, Roussel Uclaf Causality Assessment Method; TCR, T Cell Receptor; UDCA, Ursodeoxycholic acid; VBDS, Vanishing Bile Duct Syndrome.

Abstract

Drug-induced liver injury includes a spectrum of pathologies, some related to the mode of injury, some to the cell type primarily damaged. Among these, drug-induced bile duct injury is characterized by the destruction of the biliary epithelium following exposure to a drug. Most of the drugs associated with bile duct injury cause immune-mediated lesions to the epithelium of interlobular ducts. These share common histopathological features with primary biliary cholangitis, such as inflammation and necrosis at the expense of cholangiocytes and, if the insult persists, bile duct loss and biliary cirrhosis. Some drugs selectively target larger ducts. Such injury is often dose-dependent and thought to be the result of intrinsic drug toxicity. The histological changes resemble those seen in primary sclerosing cholangitis. This overview focuses on the clinical and pathological features of bile duct injury associated with drug treatment and on the immunological and biochemical effects that drugs exert on the biliary epithelium.

1. Introduction

Drug-induced liver injury (DILI) has become the leading cause of acute liver failure and transplantation in Western countries. Hepatotoxicity can be intrinsic (dose-dependent) or idiosyncratic, the latter implying that the risk of acute liver failure as a result of an allergic reaction to a given drug is usually less than 1 in every 10'000 to 100'000 exposed patients [1, 2]. DILI includes many different forms of hepatic damage, some related to the mode of cellular injury and some to the liver cell type primarily damaged [3-6].

Drug-induced bile duct injury refers to a subcategory of idiosyncratic cholestatic or mixed type (hepatic and cholestatic) injury, characterized by severe damage to the biliary epithelium and often accompanied by the disruption of the biliary tree architecture. The biliary tree is a network of intra- and extrahepatic conduits, along which the bile fluid is transported from the canaliculi into the intestine. In humans, bile ducts are categorized by size and proximity to hepatocytes. The smallest (diameter< 15 µm) is the ductule, emerging from the canal of Hering, the anatomic and physiologic transition from canaliculus to ductule. The small bile duct group includes ductules, interlobular ducts and septal ducts. Septal ducts drain into area ducts, the first of the large ducts. Area ducts, segmental ducts and the right and left hepatic ducts are intrahepatic large bile ducts. The extrahepatic part of the biliary tree includes the common hepatic duct, the cystic duct, the gallbladder and the common bile duct [7]. Cholangiocytes are the cardinal cell type of the biliary tree: they line the lumen of the bile duct and form tight junctions to seal the biliary epithelium. Cholangiocytes participate in bile formation and its regulation. They promote biliary defense against microbes ascending from the intestine, but can also be the target of immune-mediated injury [8, 9].

Depending on the site of injury, drug-induced bile duct injury in most cases affects the biliary epithelium of interlobular ducts (e.g. amoxicillin/clavulanic acid, carbamazepine), thereby sharing common pathophysiological features with other cholangiopathies. An inflammatory response directed predominantly at cholangiocytes is usually associated with prolonged cholestasis that can progress to bile duct degeneration and loss [8, 10, 11]. For the sake of clarity, such injury will be referred to as drug-induced cholangiopathy in this review; however, other definitions include: "drug-induced vanishing bile duct syndrome (VBDS)", "drug-induced bile duct injury",

“drug-induced ductopenia” and “disappearing intra-hepatic bile ducts”. Some drugs (e.g. fluorodeoxyuridines, 5-fluorouracil) selectively damage larger ducts (e.g. the common hepatic duct). Such injury is dose-dependent and thought to be the result of the intrinsic toxicity of certain drugs with a minor immune component [12]. Because the histological changes resemble those seen in primary sclerosing cholangitis (PSC), it will be referred to as drug-induced sclerosing cholangitis [10].

2. Drug-induced cholangiopathy

Signs of bile duct damage are quite common in patients with DILI, as a result of an acute phase of hepatitis; bile duct loss is less common and reflects the chronicity stage of the disease [12]. In one prospective study from Iceland, no bile duct loss cases were identified out of 96 patients diagnosed with DILI [13]. The US Drug-Induced Liver Injury Network (DILIN) recently reported that 26 of 363 (7%) patients with DILI undergoing liver biopsy presented with different degrees of bile duct loss [4]. Amoxicillin/clavulanic acid was among the most frequent causes of DILI in both studies. The incidence of amoxicillin/clavulanic acid induced bile duct loss in the DILIN study was 9% (3/34), with no cases in the Icelandic population [4, 13]. Because a number of tertiary referral centers were involved, an editorial view considered it likely that the DILIN study recruited more severe cases than would be seen in population-based studies, which could explain the higher incidence of drug-induced cholangiopathy, compared with the study from Iceland [14]. Moreover, ethnicity and genetic variability are less heterogeneous in Iceland [15]. In fact, within the DILIN study, the most severe cases tended to be associated with African-American race [4]. Moreover, the two studies used different methods to assess DILI causality: the Iceland study used the Roussel Uclaf Causality Assessment Method (RUCAM), whereas the DILIN study used a structured expert opinion process [4, 13].

2.1. Diagnosis

Current diagnosis of drug-induced liver injury is based on the internationally harmonized RUCAM approach, the method of choice to confirm or exclude the suspicion of DILI and classification of the type of injury (hepatocellular, cholestatic or mixed) based on the ratio between alanine transaminase (ALT) and alkaline

phosphatase (ASP) [16, 17]. RUCAM is validated by cases with positive rechallenge and assigns a numerical value to clinical, biochemical and serological features combined with exclusion of non-drug causes. The total score reflects the likelihood that the hepatic injury is due to a specific medication. Causal relationship is divided into highly probable (≥ 9), probable (6-8), possible (3-5), unlikely (1-2), excluded (≤ 0) [17, 18]. A recent update of RUCAM accounts for issues such as alcohol use and exclusion [19].

Drug-induced cholangiopathy usually manifests with fatigue and upper abdominal pain, coupled with dark urine and pale stools; in some instances an acute suppurative cholangitis pattern with fever, shivering and upper abdominal pain preceding the occurrence of jaundice can be observed [12]. A cholestatic phase may or may not follow and often depends on the time between the initiation of the drug and the onset of the symptoms. This time interval is highly variable, making the duration, severity and progression of the cholestatic phase unpredictable. In the DILIN study, the time of onset of symptoms after starting the medication ranged from 3 to 551 days [4]. Extrahepatic manifestations of intolerance, such as rash, eosinophilia or systemic symptoms (DRESS or Stevens-Johnson syndrome) support the diagnosis [4]. The absence of cholestatic jaundice, pruritus or xanthomas practically rules out bile duct loss [10, 14].

The laboratory markers at onset show cholestatic liver disease with very high serum alkaline phosphatase and gamma-glutamyl transferase levels, as well as high serum concentrations of bilirubin, bile acids and cholesterol. Serum aminotransferase activities usually show only minor elevations [4, 10, 12].

The histological changes are variable, depending on the severity of the lesions and the stage of disease progression. Histopathological features in the acute stage are: (i) periportal mixed inflammatory infiltrates, with poly-nuclear neutrophils and eosinophils as well as lymphocytes; (ii) alterations of the interlobular bile ducts, such as cholangiocyte swelling, cytoplasmic vacuolization, irregular distribution and size of cell nuclei, mitotic figures and ductular infiltration by lymphocytes; (iii) hepatocellular and canalicular bilirubinostasis (figures 1 A and B). The chronic stage is associated with (i) marked bile duct degeneration and loss; (ii) some portal inflammation and fibrosis; (iii) cholestasis (e.g. cholate stasis) as well as ductular reactions (figures 1 C and D). Bile duct loss is diagnosed by estimating the percentage of portal tracts devoid of

interlobular ducts or by calculating the ratio of interlobular ducts to the number of portal tracts, which in the normal liver has a value between 0.9 and 1.8 [10]. VBDS is defined as a loss of at least half of the interlobular ducts or a bile duct to portal tract ratio of less than 0.5 [10, 12]. Drug-induced cholangiopathy preferentially affects the smallest interlobular ducts with a diameter of about 30 μm or less. These lesions can be easily missed. Immunostaining for tissue polypeptide antigen (TPA) or cytokeratins such as CK7 and CK19 is often useful to better assess bile duct epithelial cells. Foci of neo-ductular proliferation can frequently be observed, with prominent cholestasis [10, 12].

2.2. Therapy and outcome

The natural history of drug-induced cholangiopathy is largely unpredictable. The bile duct loss may be of a variable extent, reversible or degenerative leading to liver failure or malignant transformation. The resolution of jaundice and cholestasis is likely due to a functional ductular proliferation driven by the small cholangiocytes, which can create alternative routes to drain the bile when the normal bile duct architecture is compromised [20]. In the DILIN study, patients with bile duct loss had an unfavorable prognosis with a higher mortality rate (27% vs. 9%; P=0.01) and chronicity rate - defined as abnormal liver tests at 6 months of follow-up (94% vs. 47%; P<0.001) - than those without bile duct loss. Among the patients with bile duct loss, the degree of loss was the best predictor of outcome. A moderately severe to severe degree of bile duct loss was associated with a poor outcome. The average percentage of portal areas with bile ducts in patients with a benign outcome was 64% compared to only 17% in those with a poor outcome (P=0.003) [4, 21].

The management of drug-induced cholangiopathy is mainly limited to the treatment of symptoms and the consequences of prolonged cholestasis. Due to its idiosyncratic nature, no randomized clinical studies can be conducted and the treatment options rely on the beneficial effect observed in other types of cholangiopathies such as PBC. Anecdotal beneficial effects of ursodeoxycholic acid (UDCA) have been shown in cholangiopathy related to prochlorperazine [22], chlorpromazine [23] and temozolimide [24]. UDCA is known to stimulate biliary secretion of bile acids and other organic compounds, reduces the hydrophobic bile acid component of bile, inhibits the intrinsic apoptotic pathway and promotes cell survival in hepatocytes, through activation of the epidermal growth factor receptor (EGFR) [68]. A case report described clinical and

biochemical resolution of drug-induced cholangiopathies using long-term immunosuppression with low-dose mycophenolate mofetil [25]. Anecdotal success in the treatment of drug–induced cholangiopathies unresponsive to UDCA alone was reported using plasmapheresis and methylprednisolone [26, 27].

2.3. Pathophysiology

Drug-induced cholangiopathy is considered a hepatic manifestation of a T cell-mediated hypersensitivity reaction against the administered compound. Supportive of this concept are eosinophilia, a history of allergy, possible concurrent Stevens-Johnson syndrome or toxic epidermal necrolysis, a shortening of the latency period when the patient is repeatedly exposed to the drug, and lymphocyte sensitization [10]. T cells are activated by antigens presented on human leukocyte antigen (HLA) molecules to the T cell receptor (TCR). HLA class I molecules are expressed in all cells and present intracellular antigens to $CD8^+$ T cells. Extracellular proteins are taken up, processed and presented on HLA class II molecules to $CD4^+$ T cells, by professional antigen presenting cells (APCs). Antigen presentation, together with additional costimulatory molecules, leads to T cell activation. These costimulatory signals can be infectious organisms, damaged cells and pro-inflammatory cytokines. Hypersensitivity reactions upon drug exposure are considered to result from the presentation of drugs by HLA molecules, followed by T cell proliferation [28, 29].

The reason why hypersensitivity to a certain drug manifests with liver toxicity in one patient and with skin reactions in another, is probably related to the multifactorial nature of drug hypersensitivity. The chemical and structural features of the drug, the genotype of the patient and other relevant epigenetic aspects (e.g. environmental factors) are all factors likely to contribute to the development of drug intolerance. These factors have been defined as “the triangle of susceptibility to drug hypersensitivity” [30]. The combination of these factors sets up a metabolic and/or an immunologic idiosyncrasy, in which certain individuals metabolize the drug to abnormal metabolites, which can exert a direct toxic effect and/or trigger an aberrant T-cell mediated reaction in patients carrying specific immunologic features (e.g. HLA aplotype).

Idiosyncratic DILI is unpredictable and lacks reliable preclinical models. While animal models to study drug-induced skin reactions have been successfully developed,

attempts to develop animal models of DILI that involve the adaptive immune system have been largely unsuccessful [31, 32]. This likely relates to difficulties in overcoming tolerance and lack of effective oral dosing strategies that induce drug antigen-specific T-cell responses [33]. In fact drug-induced skin reactions can be studied by painting low molecular weight protein-reactive chemicals directly onto the shaved abdomen of the animal. [34-36]. Using this approach, it has been possible to reproduce amoxicillin-induced skin injury and to observe that a $CD4^+$-deficient C57BL/6 mouse strain with a mutation in the αβ gene encoding for MHC class II molecules was extremely prone to sensitization to amoxicillin [37]. Recently such a mouse model has shown promise in studying flucloxacillin immunogenicity in the liver. The T cell response was antigen-specific and not activated by other β-lactam antibiotics. Oral exposure to flucloxacillin resulted in mild elevation of alanine aminotransferase and a marked swelling of the gallbladder, as in classical cholestatic models such as bile duct ligation or lithocholic acid administration [33].

Two main concepts are thought to describe T cell stimulation by a drug: the hapten theory and the pharmacological interaction with immune receptors (p-i concept). The hapten theory postulates that chemical compounds bind covalently to endogenous proteins to form antigenic complexes that induce T cell responses. According to this model, drugs or chemicals are too small to induce an immune response on their own; but they can become immunogenic after coupling with a protein to form a hapten–protein covalent complex. To act as an antigen, the hapten–protein complex requires antigen processing and presentation [28]. The p-i concept proposes that certain drugs, albeit chemically inert and incapable of covalently binding to proteins, can still interact at low affinity with HLA molecules and prime the TCR interaction and the immune cascade [29]. Haptens (covalent) are primarily immunogenic due to their chemical features, whereas labile (non-covalent) interactions depend on the structure of the drug. The type of immune reaction might differ depending on the type of immunogenicity. The very same drug might induce labile and hapten-like presentations in different tissues or individuals [29].

2.3.1. Drug metabolism by cholangiocytes

The liver is the major organ responsible for the biotransformation of many endogenous substances (e.g. bile salts, hormones) and xenobiotics into more hydrophilic products,

and elimination through bile secretion. Typically, biotransformation occurs in phase I and phase II reactions. The most common phase I drug-metabolizing enzymes are represented by the CYP450 superfamily. During phase II drug metabolism, the metabolites from phase I pathways are enzymatically conjugated with hydrophilic endogenous compounds such as glutathione (GSH) [38]. The detoxification capacity of the liver has been mainly attributed to hepatocytes: upon transformation, drugs are excreted into the bile, exposing the cholangiocytes to high concentrations of potentially reactive species [39]. It is also possible that the very same metabolites can be produced, to a certain extent, by cholangiocytes as well. However, not much is known about the contribution of cholangiocytes to the liver's detoxification capacity. Cholangiocytes isolated from rat have a very high specific activity of gamma-glutamyltranspeptidase (200-times that in hepatocytes), enzymes related to the glutathione redox cycle (e.g. GSH-peroxidase, GSSG-reductase and different isozymes of GSH-transferase), but a very low content of GSH and a lack of CYP450 activity [40-42]. Conjugating activity was detected in primary cholangiocytes isolated from patients [43]. Interestingly, CYP1A, CYP2E1, and CYP3A transcripts and proteins were detected in human cholangiocytes. CYP2E1 exhibited a similar pattern of expression in small and large cholangiocytes, whereas CYP3A was detected only in medium and large intrahepatic ducts [43].

GSH is an important protective mechanism in the detoxification of reactive metabolites in hepatocytes [44, 45]. Low hepatocellular GSH content is a theoretical risk factor for hepatotoxicity secondary to reactive metabolites, which are quenched by GSH. The downstream effects of reactive metabolites are related to the formation of covalent protein adducts, which can inhibit or change the subcellular localization of proteins. Reactive metabolites can also act as neo-antigens (haptens) that trigger an immune response. Human primary cultured biliary epithelial cells were shown to be more sensitive than primary cultured hepatocytes to the flucloxacillin metabolite 5-hydroxymethylflucloxacillin, a product of CYP3A4-mediated oxidation. Whether this increased sensitivity is related to cholangiocellular GSH content, would need to be addressed experimentally in primary cultured biliary epithelial cells [42, 46]. Several studies have shown that flucloxacillin and 5-hydroxymethylflucloxacillin, bind and modify serum proteins in vivo (figure 2) [47, 48].

Other cholangiopathy-inducing drugs that generate hapten-protein complexes include amoxicillin/clavulanic acid, sulfamethoxazole and carbamazepine [49-53]. Unlike flucloxacillin, the metabolites of sulfamethoxazole and carbamazepine, but not the parent compounds, were shown to generate hapten-protein complexes in rodents and in microsomes from human liver, respectively [54, 55]. Based on in vitro studies in human microsomes, terbinafine, an antifungal agent used for the treatment of superficial infections and associated with idiosyncratic cholangiopathy, has also been hypothesized to generate hapten-protein complexes. The aldehyde (TBF-A) generated from the N-dealkylation at the allylic N-C bond is thought to conjugate with glutathione (GSH), to be transported across the canalicular membrane of hepatocytes and to accumulate in bile, where dissociation from GSH and binding to hepatobiliary proteins could occur [56]. The importance of GSH in hapten-protein immunogenicity has been demonstrated for sulfamethoxazole. Pharmacokinetic studies showed that sulfamethoxazole is predominantly metabolized by N-acetyl transferase and N-glucuronyl transferase to non-toxic metabolites [57]. However, a fraction of drug is N_4-hydroxylated by CYP450 enzymes to a hydroxylamine metabolite, which can be further oxidized to the nitroso compound [58, 59]. Studies in vitro demonstrated that the nitroso compound can bind to thiol groups of proteins and form hapten-protein complexes [49, 60]. Oxidation of hydroxylamine to nitroso is prevented by GSH. GSH can also react with the nitroso moiety to form an unstable semi-mercaptal conjugate that is rapidly rearranged to a stable sulfonamide metabolite or is cleaved to hydroxylamine [58]. The hydroxylamine is less prone to haptenate proteins, compared with the nitroso metabolite [60]. An important point to note is that hapten–protein complexes can be detected in all individuals exposed to the drug and that they are not unique to those developing toxicity. Analysis of albumin isolated from the serum of eight flucloxacillin-tolerant patients showed that a similar pattern of modification occurred in all patients, indicating that the presence of an adduct alone is not sufficient to cause toxicity [47].

2.3.2. Immunological features of cholangiocytes

Rather than representing a mere passive scaffold for bile to flow from the canaliculus to the intestine, cholangiocytes are now considered a highly specialized cell type, with a cardinal role in the immune response of both infectious and non-infectious hepatobiliary diseases. The biliary epithelium is exposed to an extremely unfriendly

environment at the apical membrane: high concentrations of hydrophobic bile salts, drug metabolites and foreign antigens ascending from the gastrointestinal tract continuously jeopardize the mucosal homeostasis in the biliary tract. Cholangiocytes secrete immunoglobulin A (IgA) and are equipped with an apical glycocalyx layer made of proteoglycans and glycoproteins, to limit the access of molecules of different size to the plasma membrane. Glycocalyx and HCO_3^- provide an important physicochemical barrier that protects the apical domain of cholangiocytes against hydrophobic bile acids and other toxins contained in bile [61, 62]. Cholangiocytes express a variety of Toll-like receptors (TLRs) that are activated by pathogens and initiate a signal cascade to recruit T cells, macrophages and natural killer cells to resolve biliary infections. It has been hypothesized that TLR plays an essential role in discriminating between self and non-self, thus preventing the development of autoimmune conditions. Indeed, TLR expression and genetic variants (e.g. single nucleotide polymorphisms) have been correlated with the development of certain autoimmune conditions [63].

HLA molecules are key proteins that regulate T cell-mediated immunity. In fact, genetic HLA variation is particularly relevant for the development of hypersensitivity reactions, including idiosyncratic drug-induced cholangiopathy [64]. Cholangiocytes constitutively express HLA class I molecules. In cholestatic disease and during liver allograft rejection, cholangiocytes express HLA class II molecules as well, suggesting an APC-mode activity [65-67]. Nonetheless, due to the absence of costimulatory molecules such as CD80 and CD86, it is unclear whether cholangiocytes can actually function as APC [68, 69]. It has been suggested that the main interaction between cholangiocytes and T cells is through other molecules, such as leukocyte factor antigen-3/CD2 and CD40/CD40L [70]. Healthy cholangiocytes express CD1d at the basolateral side [71]. CD1d controls the function of natural killer T cells (NK T). The physiological role of NK T cells is considered to be immunoregulatory. Lipids are natural ligands of CD1d. Activated NK T secrete large amounts of interleukin-4 (IL-4) without prior priming with this cytokine and can bestow a type 1 or type 2 differentiating potential upon $CD4^+$ helper T lymphocytes [72]. On the apical side, cholangiocytes are exposed to the lipid constituents of bile and it is possible that lipid antigens can be taken up by cholangiocytes, presented by basolaterally localized CD1d, to potentially trigger immune reactions by NKT cells in the surrounding liver tissue [71]. CD1d is also thought to function as a sensor, sensing alterations in cellular lipid content by virtue of its affinity for such ligands. The presentation of a neo-self glycolipid, presumably

induced by foreign antigens, activates NK T cells. Activated NK T cells promptly release pro-inflammatory cytokines (e.g. IL-4) to jump-start the immune system [72]. This complex “arsenal” of innate and adaptive immune responses predispose cholangiocytes to immune-mediated attack, especially in genetically susceptible individuals who experience an exaggeration of cholangiocyte responses, resulting in an enhanced, uncontrolled pro-inflammatory response.

Most of the drugs causing bile duct loss have been associated with HLA predisposition, supporting a probable role for activated T cells in the molecular mechanisms of liver damage (table 1). A study of 35 cases of amoxicillin-clavulanate induced cholangiopathy, assessed by the RUCAM score, demonstrated that the DRB1*1501-DRB5*0101-DQB1*0602 HLA haplotype was significantly more frequent in patients than in controls (57.1% vs 11.7%) [73]. Shortly thereafter, results from a study on 140 patients with a definite or probable diagnosis of DILI, as assessed by RUCAM, and 635 volunteers, demonstrated an HLA-DRB1*15 and -DQB1*06 allele association with cholestatic/mixed hepatic damage, regardless of the causative drug [74]. A genome-wide association study from patients diagnosed with DILI according to RUCAM identified the HLA-B*57:01 allele as a risk factor for flucloxacillin-induced liver injury. Among flucloxacillin DILI cases, 85% carried the risk allele. Carriers of the HLA-B*57:01 allele have an 80-fold increased risk for developing liver toxicity on flucloxacillin treatment, although the absolute risk still remains < 1:500 [75]. Another genome-wide association study from patients diagnosed with DILI found that individuals carrying the HLA-A*33:01 have a 40-fold higher risk of developing terbinafine-induced liver injury [64]. A recent study using peripheral blood mononuclear cells (PBMC) from patients treated with amoxicillin/clavulanic acid demonstrated that drug hapten-responsive $CD4^+$ and $CD8^+$ T cells were detectable, but only in the presence of professional APCs [76]. This finding indirectly supports the observation that in cholestatic conditions, cholangiocytes might act very much as APC, triggering T cell activation. Nevertheless, which costimulatory molecules are possibly involved in the jump-start of the immune response remains an enigma. While amoxicillin monotherapy is well-tolerated, the combination with clavulanic acid increases the risk of cholangiopathy, suggesting that the latter might be responsible for the immune response [77]. Mass spectrometry studies in blood samples from patients exposed to amoxicillin/clavulanic acid showed that both drugs are capable of haptenization and unique antigen formation. It is possible that the addition of clavulanic acid increases

the chemical repertoire of antigenic structures in patients, which in turn may increase the possibility of immune recognition in a HLA restricted manner [53]. Establishing the immunogenicity of amoxicillin and clavulanic acid in the presence of the DRB1*1501-DRB5*0101-DQB1*0602 HLA haplotype would shed some light on this matter.

While the amoxicillin/clavulanic acid immune response is considered to be mainly driven by hapten-protein complex generation, drugs such as flucloxacillin, sulfamethoxazole and carbamazepine undergo both labile and hapten-like presentations [78-80]. In vitro, sulfamethoxazole and its nitroso metabolite interact differently with the TCR, with the former having a labile interaction and the latter a hapten-like interaction [80]. Ex vivo experiments demonstrated that: (i) flucloxacillin hapten-protein complexes are presented by various HLA molecules and mainly induce $CD4^+$ T cell proliferation; and (ii) in HLA-B*57:01 individuals flucloxacillin mainly interacts in a noncovalent fashion with the HLA and TCR receptors (p-i concept), leading to the expansion of $CD8^+$ T cells and secretion of interferon-γ and cytolytic molecules (figure 3) [78, 81]. One type of interaction might predominate over another, depending on the genetic profile of the individual and the site of immunogenicity, resulting in a variable clinical manifestation of the hypersensitivity. One may speculate that flucloxacillin-induced cholangiopathy is mainly driven by the noncovalent interaction between the HLA-B*57:01 variant, the drug and the TCR. However, it should be emphasized that not all patients with flucloxacillin-induced cholangiopathy express HLA-B*57:01, and drug-specific, HLA-restricted T-cell responses are also detectable in HLA-B*57:01 negative individuals.

3. Drug-induced sclerosing cholangitis

Drug-induced sclerosing cholangitis refers to one or more strictures of the large bile ducts, mainly the common hepatic duct and the right and left hepatic ducts, usually sparing the common bile duct and the smaller intrahepatic ducts [10, 12]. Hepatic artery infusion of a fluoropyrimidine (e.g. 5-fluorouracil, fluorodeoxyuridine), an effective therapeutic option in patients with liver-restricted or liver-predominant metastatic colon cancer who have progressed while on first-line chemotherapy, is the main treatment associated with sclerosing cholangitis, with an incidence up to 1 out of 5 treated patients [82-85].

Sclerosing cholangitis is a relatively frequent complication of scolicidal solutions. Hydatid disease is a parasitic infestation characterized by cysts. The most common site for a hydatid cyst is the liver and the biliary ducts. Scolicidal agents (hypertonic saline 20%, povidone iodine 1%, silver nitrate 0.5% or 5% formalin) are injected into the cyst to deactivate the scolices. A communication between the cyst and the biliary tree, prolonged exposure of the scolicidal agent to the biliary tree, and a particular sensitivity to the scolicidal agent might concur to promote caustic sclerosing cholangitis [86].

Ketamine abuse has been postulated to induce sclerosing cholangitis. Ketamine abusers can experience epigastric pain and elevated serum alkaline phosphatase and gamma-glutamyl transferase, often associated with dilatation of the common bile duct [87-90].

Analysis of potential bile duct abnormalities in a large unselected patient group with DILI showed that drugs usually associated with cholangiopathy (e.g. amoxicillin-clavulanic acid) can also induce sclerosing cholangitis. Interestingly, all patients with cholangiographic abnormalities (10/25) were female [91]. Because sclerosing cholangitis was diagnosed by imaging, no information as to whether the damage was immune-mediated was obtained. The cholestatic phenotype of the sclerosing cholangitis group was more severe (more patients with jaundice and hospitalization) than that of the other cholestatic DILI cases. The time to resolution of liver tests was also significantly prolonged in the patients with sclerosing cholangitis. Nonetheless, the patients with sclerosing cholangitis displayed a relatively good prognosis [91].

3.1. Diagnosis

The clinical feature of large bile duct injury at the acute stage is transient cholangitis, which is followed after months by worsening cholestasis as a result of biliary sclerosis. Symptoms include upper abdominal pain, jaundice, anorexia and weight loss. Continuous irregularities with intra- and/or extrahepatic bile duct dilatation can be seen in magnetic resonance cholangiopancreatography [91]. The histopathological changes are not specific, and correspond to parenchymal and periportal features of chronic cholestasis. They resemble the changes seen in primary sclerosing cholangitis: fibrous

obliterating cholangitis, with varying degrees of duct atrophy and involution, eventually resulting in ductopenia [10, 12].

3.2. Therapy and Outcome

The outcome is variable: most cases appear to be more or less reversible; some develop hepatic failure [82, 84, 92]. In chemotherapy-induced sclerosing cholangitis, the development of strictures can usually be prevented by dose reduction or stopping the treatment when increased liver function tests are noted. The routine addition of intra-arterial steroids and selective use of UDCA are also helpful. Severe strictures causing jaundice may require biliary stenting [84].

3.3. Pathophysiology

Because of the high hepatic uptake of fluoropyrimidine administered via the hepatic artery, systemic toxicity is minimal but bile duct damage is often accompanied by abnormalities in the blood flow scan, indicating that the injury is probably of ischemic nature. Ischemia is likely to be secondary to the microvascular supply of the bile ducts by the drug itself, rather than a complication of the surgical insertion of the intra-arterial catheter. When other chemotherapeutic agents like cisplatin, oxaliplatin or irinotecan were used, sclerosing cholangitis complications were essentially absent [93-96]. In animals, 5-fluorouracil induced disruption of the endothelial sheet and patchy exposure of the subendothelium as a matrix for thrombus formation [97]. *Ex vivo* and *in vivo* polarographic analysis of erythrocytes showed a rapid depletion of pO_2 in erythrocytes exposed to 5-fluorouracil, likely resulting in an increased production of 2,3-bisphosphoglycerate, which leads to further deoxygenation and to the increase of the level of deoxyhemoglobin. This, in turn, changes rheological membrane properties and leads to ionic misbalance in erythrocytes. All these changes diminish the ability of erythrocytes to deliver oxygen to the tissues [98].

The mechanism by which ketamine abuse leads to cholestasis and biliary dilatation has not been studied. Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist. The activation of NMDA on smooth muscle cells is responsible for the contraction of the human ureter. Ketamine-induced smooth muscle relaxation might explain the occurrence of hydronephrosis in ketamine abusers. It has been

speculated that this effect also extends to the biliary tree, thus causing biliary dilatation [88].

4. Summary and Outlook

While drug-induced large bile duct injury is a predictable side effect of certain therapeutic regimens, manageable and usually associated with a good outcome, drug-induced damage of small bile ducts is rare, complex, mostly unpredictable and potentially fatal. While a large amount of information regarding the chemistry and pharmacology of a candidate drug is gathered during the drug development process, very little is usually known about the genetic and epigenetic features of the patient(s). Although high throughput assays exist to evaluate potentially toxic molecules based on *in vitro* covalent binding, these assays alone are inadequate to predict the potential of a candidate drug and/or its metabolite(s) to induce drug-induced cholangiopathy or, more generally, drug-induced hypersensitivity. Hence, there is a need to develop assays that can integrate the various risk factors and enhance the predictability of drug hypersensitivity. As described above, certain drugs such as flucloxacillin can induce the immune response due to structural characteristics and interaction with immune receptors (HLA and TCR). This feature has not yet been considered in the development of a drug, but may account for a substantial portion of unforeseen side effects [29].

While studies in animal models have been useful in predicting intrinsic DILI [99], attempts to develop animal models of idiosyncratic DILI that involve the adaptive immune system have been largely unsuccessful. Recently, a $CD4^+$-deficient mouse carrying a mutation in the $\alpha\beta$ gene encoding for major histocompatibility complex (MHC) class II molecules, has been shown to be a promising model to study $CD8^+$-restricted flucloxacillin immunogenicity. The liver became cholestatic upon a T-cell response specific against flucloxacillin but not against other β-lactam antibiotics [33]. Characterization of the molecular pathophysiological mechanism(s) of drug-induced cholangiopathy combining *in vitro* assays and animal models with retrospective gene analyses of susceptible patients is a critical step towards more reliable prediction of drug-induced cholangiopathy during the drug development process.

Table 1. Drugs associated with bile duct injury and immunogenic susceptibility.

Drug	Cohort	Association	Ref.
Amox/Clav *	35 (European)	DRB1*1501 #	[73]
Flucloxacillin *	51 (European)	HLA-B*5701 #	[75]
Terbinafine ¥	14 (mixed)	HLA-A*3301 #	[64]

* Causality assessed by RUCAM
¥ Cases recruited by the international Drug-Induced Liver Injury Consortium (iDILIC)

Figure 1. Histology of the portal tract in a patient with probable (updated RUCAM score=8) amoxicillin/clavulanic acid-induced DILI (A and B). A (H&E): Inflammatory infiltrate with abundant eosinophils. The interlobular bile duct (black arrowhead) with irregular and vacuolated epithelium, infiltrated by lymphocytes. Periportal liver parenchyma with intracellular and canalicular bilirubinostasis (white arrowheads). **B** CK7 immunohistochemistry highlights the interlobular bile duct and the extensive ductular reaction. Scale bar 50 μm. **Portal tract in a patient with possible (updated RUCAM score=4) DILI after NSAID treatment (C and D). C** (H&E): clearly distinguishable branches of the liver artery (black arrowheads) and the portal vein (white arrowhead). Morphologically, there is scant inflammatory infiltrate and loss of the interlobular bile duct. **D** Minimal ductular reaction in the CK7 stain, but no true interlobular bile duct. Scale bar 100 μm.

Figure 2. Hapten–protein complexes formed by flucloxacillin. Flucloxacillin is oxidized by CYP3A4 to 5-hydroxymethylflucloxacillin. Both parental and metabolite compounds can covalently bind to protein lysine residues.

Figure 3. HLA haplotype and T cell reactivity to flucloxacillin. Flucloxacillin is presented in a labile (p-i interaction) or in a covalent (hapten-protein interaction) manner to the T cell receptor (TCR). When the interaction is labile, $CD8^+$ T cell proliferation is restricted to the HLA-B*57:01 allele. Flucloxacillin hapten-protein complexes induce mainly $CD4^+$ T cell proliferation and the stimulation is HLA-unrestricted. Flucloxacillin hapten-protein complexes are poorly immunogenic, in the presence of the HLA-B*57:01 variant.

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